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Review

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ABSTRACT

The emergence of multiple strains of bluetongue virus (BTV) and the recent discovery of Schmallenberg virus (SBV) in Europe have highlighted the fact that exotic *Culicoides*-borne arboviruses from remote geographic areas can enter and spread rapidly in this region. This review considers the potential for this phenomenon to impact on human health in Europe, by examining evidence of the role of *Culicoides* biting midges in the zoonotic transmission and person-to-person spread of arboviruses worldwide. To date, the only arbovirus identified as being primarily transmitted by *Culicoides* to and between humans is Oropouche virus (OROV). This member of the genus *Orthobunyavirus* causes major epidemics of febrile illness in human populations of South and Central America and the Caribbean. We examine factors promoting sustained outbreaks of OROV in Brazil from an entomological perspective and assess aspects of the epidemiology of this arbovirus that are currently poorly understood, but may influence the risk of incursion into Europe. We then review the secondary and rarely reported role of *Culicoides* in the transmission of high-profile zoonotic infections, while critically reviewing evidence of this phenomenon in endemic transmission and place this in context with the presence of other potential vector groups in Europe. Scenarios for the incursions of *Culicoides*-borne human-to-human transmitted and zoonotic arboviruses are then discussed, along with control measures that could be employed to reduce their impact. These measures are placed in the context of legislative measures used during current and ongoing outbreaks of *Culicoides*-borne arboviruses in Europe, involving both veterinary and public health sectors.

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1. Introduction

Culicoides biting midges (Diptera: Ceratopogonidae) are among the smallest blood-sucking flies, with body lengths that rarely exceed three millimeters (Mellor et al., 2000). The developmental cycle of *Culicoides* consists of egg, four larval instars, pupa and adult (Fig. 1). Almost all *Culicoides* require moisture-rich habitats for development of egg, larval and pupal forms and the availability of these environments is a key determinant limiting distribution, abundance and seasonal occurrence (Mellor et al., 2000). Almost 1400 extant and extinct species of *Culicoides* have been described from a highly diverse range of ecosystems and the genus is present on all major land masses with the exception of Antarctica and New Zealand (Borkent, 2004, 2013; Mellor et al., 2000). Only an extremely small proportion of *Culicoides* species have a significant deleterious impact on human existence, however, even in the case of species known to play a role in transmitting arboviruses, bacteria, protozoa and helminth parasites to humans and animals, they remain the least studied of the major Dipteran vector groups. This is in part a consequence of their small size and fragility, which imposes significant limitations on studies of their ecology and for the most part prevents laboratory colonization of vector species, but is also due to the limited direct impact of *Culicoides* on human health.

In light of the recent and unprecedented emergence of Schmallenberg virus (SBV), a novel arbovirus of ruminants that upon discovery had unknown zoonotic potential, we assess the potential for *Culicoides* midges to act as vectors of human-to-human transmitted or zoonotic arboviruses in Europe. This review complements

earlier papers that have discussed the role of *Culicoides* in public health worldwide (Linley et al., 1983) and as vectors of arboviruses (Mellor et al., 2000) and non-viral pathogens (Linley, 1985). No attempt is made to discuss in detail biological transmission of arboviruses by *Culicoides* for which recent reviews are already available (Mellor et al., 2009; Tabachnick, 2013) and the role of *Culicoides* in the emergence of livestock arboviruses in Europe (Carpenter et al., 2009; MacLachlan and Mayo, 2013; Mellor and Wittmann, 2002; Purse et al., 2005) is considered only where relevant to the primary subject (and summarized briefly in Table 1).

2. Current status of *Culicoides* and public health worldwide

Globally, the most commonly observed impact of *Culicoides* biting midges on public health occurs through nuisance biting inflicted by female adults, leading in severe cases to cutaneous pruritic wheal-and-flare responses and permanent scarring (Felippe-Bauer and Sternheim, 2008; Linley et al., 1983; Sherlock, 1965). Opportunistic feeding on humans by a wide range of *Culicoides* species has been documented; however, certain species have become notorious for this activity through their vast population density and persistent biting attacks, shaping public perception of the genus in many regions including northern Europe. *Culicoides* that inflict biting nuisance have been investigated in greatest detail where they impact tourism, forestry and agriculture (Hendry, 2011; Hendry and Godwin, 1988; Linley and Davies, 1971).

Despite this record of biting nuisance and their role as vectors of internationally important arboviruses of livestock (Mellor et al.,

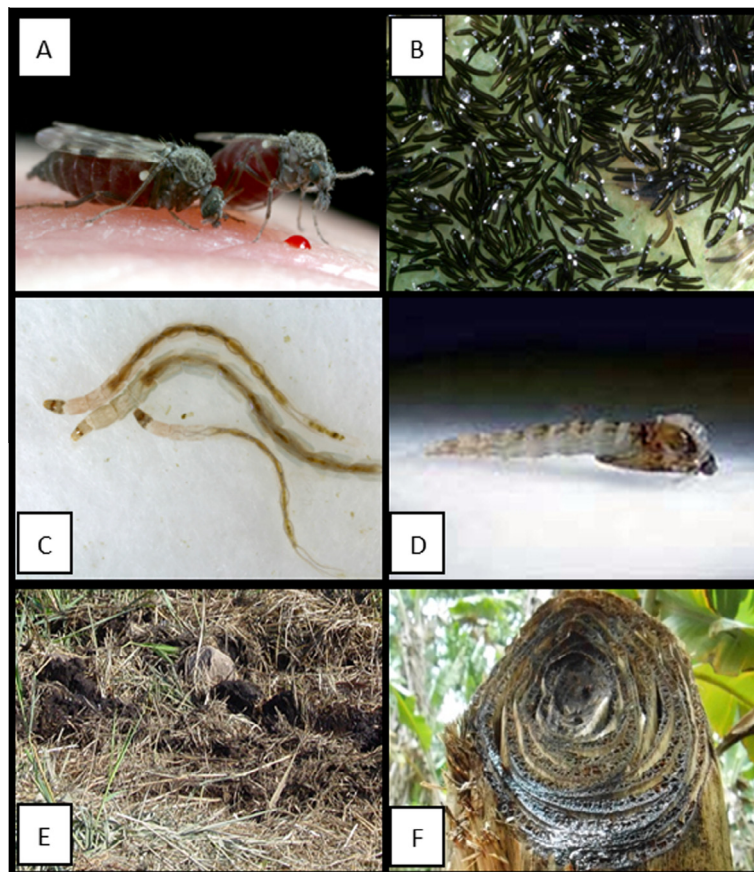


Fig. 1. Life cycle and developmental substrates of *Culicoides* biting midges. (A) Blood-feeding adult female *Culicoides nubeculosus* on a human host (wing length ≤ 2 mm); (B) Cigar-shaped eggs (length ≤ 2 mm); (C) Active, semi-aquatic larvae with vermiform body shape (length ≤ 5 mm); (D) Largely inactive pupal stage (length ≤ 5 mm); (E) Larval development habitat of *C. imicola* in Corsica, France (organically enriched soil); (F) Larval development habitat of *C. paraensis* in Brazil (rotting banana stem). Figs. (A–D) courtesy of The Pirbright Institute; Fig. E courtesy of Cirad; Fig. F courtesy of Instituto Oswaldo Cruz.

Table 1
Characteristics of arboviruses transmitted by *Culicoides* biting midges.

Bluetongue virus	<p><i>Orbivirus</i></p> <p>Identified as a filterable agent during the early 20th century in South Africa</p> <p>Infects all ruminant species investigated to date and surveys of antibody prevalence have identified additional host species that may also be able to sustain transmissible viraemia</p> <p>Clinical disease is most severe in sheep and deer with milder signs in cattle. Economic impact can be huge: total cost of the BTV-8 incursion in Europe is likely to exceed 1000 million Euros</p> <p>Virtually worldwide distribution between latitudes 35°S to 45°N including temperate regions with seasonal absences of <i>Culicoides</i> adults</p> <p><i>Culicoides</i> act as the primary biological vector and involvement of other vectors is thought to be epidemiologically negligible</p> <p>No evidence of animal-to-human transmission</p>
Schmallenberg virus	<p><i>Orthobunyavirus</i></p> <p>Identified using metagenomic sequencing during 2011 in Germany</p> <p>Infects cattle, sheep and other ruminants. Antibodies have also been identified in deer.</p> <p>Clinical disease in sheep and cattle characterized by congenital deformities in young born to adults infected in their first trimester. Economic impact relatively limited</p> <p>Detected in Palearctic region only (although presumed to be of tropical origin)</p> <p><i>Culicoides</i> are the only vector identified to date</p> <p>No evidence of animal-to-human transmission</p>
Oropouche virus	<p><i>Orthobunyavirus</i></p> <p>Identified by isolation during 1955 in Trinidad.</p> <p>Host range is poorly characterized but antibodies to infection have been recorded from a range of distantly related mammals.</p> <p>Primary disease impact is in humans as a mild febrile illness with rare incidence of more serious clinical disease. Impact on livestock and companion animals unknown</p> <p>Detected in neotropical region only</p> <p><i>Culicoides</i> and several species of <i>Culicidae</i> (mosquitoes) identified as vectors for human-to-human transmission</p>

2000), *Culicoides* have only rarely been implicated as the primary agents of pathogen transmission to or between humans. Exceptions to this include a range of filarial nematodes transmitted between humans, most notably *Mansonella ozzardi*, *M. perstans* and *M. streptocerca* (Linley et al., 1983) which are of high prevalence in Latin America and the Caribbean (Hawking, 1979) and west and central Africa (Simonsen et al., 2011). Because the clinical manifestation of mansonellosis is commonly either mild or entirely asymptomatic, examinations of the epidemiology of transmission by *Culicoides* are relatively rare. A notable exception are the series of detailed investigations defining relative roles of *Culicoides* and blackflies (Diptera: Simuliidae) in transmission of *M. ozzardi* in South America (Shelley and Coscaron, 2001; Wirth and Felipe-Bauer, 1989; Yarzabal et al., 1985).

By far the most important current role of *Culicoides* biting midges in public health lies in their ability to biologically transmit Oropouche virus (OROV), the aetiological agent of the febrile illness Oropouche fever, between human beings (Linley et al., 1983; Mellor et al., 2000). Commonly observed symptoms of Oropouche fever include headache in a high proportion of cases, but can also lead to generalized arthralgia, anorexia and in rare cases meningitis, the incidence of which remains undetermined in the vast majority of epidemics (LeDuc and Pinheiro, 1989). OROV is widely distributed across a geographic range that is thought to include Brazil, Peru, Panama, Colombia and Trinidad (Karabatos, 1985; Nunes et al., 2007; Saeed et al., 2000), but has not to date been recorded in nearby Costa Rica, Venezuela or other Caribbean islands. Major OROV disease epidemics have largely centered upon Brazil (Pinheiro et al., 1962; Vasconcelos et al., 1989, 2009, 2011), where thousands of clinical cases can occur and yearly incidence in humans is thought to be surpassed only by dengue among arboviral pathogens, although the lack of specificity of clinical symptoms, combined with a high background of febrile illnesses, hampers accurate reporting. The distribution and incidence of endemic OROV is also currently under review in light of the recent discovery in Peru of Iquitos virus, which has similar clinical manifestations, but whose mode of transmission has yet to be investigated in detail (Aguilar et al., 2011).

It has been hypothesized that OROV persists in sylvatic endemic cycles of transmission, although these remain poorly characterized and may involve multiple vectors and reservoir hosts (Pinheiro et al., 1981a). Investigation of candidate vector(s) has centered upon mosquitoes, but although isolations of OROV have been made from *Aedes serratus* and *Coquillettidia venezuelensis* (Anderson et al., 1961; Pinheiro et al., 1981a), the number of successful recoveries of the virus has been extremely low. The challenge of making positive isolations of OROV from adult vectors under endemic scenarios is illustrated by the isolation of only a single strain of the virus from processing over 1 million mosquitoes, phlebotomine sandflies, ticks and other ectoparasites in the Amazon region during inter-epidemic periods (Pinheiro et al., 1981a). Screening of potential reservoir hosts for OROV has also been undertaken but remains inconclusive, with antibodies to infection detected in a wide range of domestic and wild avian species, primates, wild carnivores and rodents (Batista et al., 2012; Pinheiro et al., 1981a). Isolations of OROV, that may be indicative of a transmissible viraemia, have also been made from a sloth *Bradypus tridactylus* (Pinheiro et al., 1962) and a sylvatic monkey *Callithrix* sp. (Nunes et al., 2005). Replication and concurrent clinical signs also occur in the golden hamster (*Mesocricetus auratus*), which is currently used as an experimental model (Pinheiro et al., 1982; Rodrigues et al., 2011). Interestingly, the ability of OROV to replicate in livestock appears not to have been addressed in studies to date, as major outbreak areas of disease have not coincided with centers of ruminant production.

In contrast to the theoretical sylvatic cycle, epidemic transmission of OROV between humans as an anthroponosis are well characterized, being driven almost exclusively by *C. paraensis*. The role of this species as a vector of OROV has been investigated in both the field (Roberts et al., 1981) and in the laboratory (Pinheiro et al., 1982, 1981b). The latter studies are notable for convincingly demonstrating biological transmission of OROV between hosts by *Culicoides* and are among the most complete vector competence trials of the genus. Larvae of *C. paraensis* develop in microhabitats of decomposing banana and plantain stalks and stumps and cacao hulls (Hoch et al., 1986) (Fig. 1F), having originally exploited rotting organic material in dry tree-holes, leaf debris and damp soil for this purpose (Mercer et al., 2003; Pappas et al., 1991; Wirth and Felipe-Bauer, 1989). Following fruit harvesting, these waste products accumulate in close proximity to high-density human housing, resulting in biting attacks of *C. paraensis* adult females on inhabitants.

Unlike the majority of other *Culicoides* species that have a primarily crepuscular (dusk and dawn) periodicity (Kettle, 1977; Mellor et al., 2000), *C. paraensis* is also active diurnally with only a slight apparent peak in host-seeking female activity in the late afternoon (Mercer et al., 2003; Roberts et al., 1981). Hence, while the maximum biting rate recorded for *C. paraensis* is a relatively modest 14.4 adults collected/min on a human subject in Belém, Brazil (Hoch et al., 1990), their diurnal activity combined with a propensity to enter human housing (Roberts et al., 1981), leads to a consistent low level of biting in both day and night. Quantifying this biting activity and the resulting impacts on transmission is therefore difficult when using a standard 'snapshot' estimate of crepuscular *Culicoides* abundance. Importantly, these rates appear to be insufficient to trigger changes in human behavior to combat the nuisance and reduce OROV transmission, despite the fact that systematic clearing of larval habitats has been shown to be effective in reducing *C. paraensis* numbers (Hoch et al., 1986).

In addition to OROV, *Culicoides* may also play a limited but poorly defined role in the transmission of many other zoonotic arboviruses of global importance (Table 2). By far the best characterized of these is vesicular stomatitis Indiana virus (VSIV), though research concerning the transmission of this virus by *Culicoides* has been entirely focused on ruminants (De Leon and Tabachnick, 2006; Drolet et al., 2005; Nunamaker et al., 2000). This is because cases of human disease arising from arthropod transmission of VSIV are thought to be extremely rare (Krauss et al., 2003; Letchworth et al., 1999). Of the other human pathogenic arboviruses that have been detected in field-caught adult female *Culicoides*, oral susceptibility has only been investigated in detail for Rift Valley fever virus (RVFV), following initial detection in field populations. In this study RVFV failed to replicate in 135 individuals of a

C. sonorensis colony line derived from a population originally colonized in the USA (Jennings et al., 1982).

3. Current status of *Culicoides* and public health in Europe

The public health importance of *Culicoides* biting midges in Europe is currently restricted to biting nuisance, largely inflicted by a single species, *C. impunctatus*. While this species is widespread and abundant in many northern European countries, including the Netherlands (Takken et al., 2008), the vast majority of detailed studies of *C. impunctatus* have centered on populations in the Scottish Highlands (Blackwell, 2001; Stuart et al., 1996). Here, the abundance of *C. impunctatus* vastly exceeds that recorded in the rest of northern Europe and can result in biting rates on humans that exceed those recorded for the vast majority of hematophagous dipteran species worldwide, with a maximum of 635 *C. impunctatus* collected/minute on human bait arms in Ormsary, UK (Carpenter et al., 2005). In contrast to *C. paraensis*, *C. impunctatus* is primarily a crepuscular species (Blackwell, 1997), although biting episodes are recorded during the day under overcast conditions or in forest cover (Hendry, 2011). A key factor driving the huge population abundance of *C. impunctatus* lies in the ability of adult females to produce eggs without taking a blood meal (autogeny) (Blackwell et al., 1992; Boorman and Goddard, 1970). This is a selectively advantageous trait in areas of low available host density, and where *Culicoides* larval development sites are consistently available (Linley, 1983). Autogeny is especially common among major nuisance species of humans, as compared to species that only take their blood meals from animals (Isaev, 1993; Linley, 1983). *Culicoides impunctatus* additionally possesses a broad host range, with evidence of feeding on a wide range of livestock and wildlife, in addition to humans (Blackwell et al., 1995, 1994a).

The larval habitat of *C. impunctatus* is well defined, consisting of rush-pasture-peat communities possessing high organic and water content (Blackwell et al., 1999, 1994c), created in part through tree clearance (Hendry, 2011). In Scotland, northern England and Wales, these bog heathland ecosystems are extensively used for recreation (Blackwell and Page, 2003), forestry and hunting, all of which can involve prolonged human exposure to biting populations of *C. impunctatus*. The economic impact of such attacks on tourism is thought to be significant, however, quantitative assessments of tolerance of individuals visiting these regions have not been carried out to date. However, anecdotal estimates from studies carried out in the Caribbean estimate that biting rates greater than 5/h may be sufficient to impact tourist behavior (Linley and Davies, 1971). Disruption of forestry in Scotland by *C. impunctatus* has been investigated, and is estimated in some areas to lead to the

Table 2

Detection of pathogenic arboviruses of humans from field-collected adult *Culicoides* midges with no visible blood meal remnants, indicating replication of the virus in the *Culicoides* host and a potential for onwards biological transmission (Mellor, 2000).

Genus	Virus	Location of study	<i>Culicoides</i> species	References
<i>Orthobunyavirus</i>	Shuni [*]	Nigeria	Mixed	(Lee, 1979)
<i>Phlebovirus</i>	Rift valley fever [#]	Kenya	Unidentified	(Davies et al., 1979)
		Nigeria	Unidentified	(Lee, 1979)
<i>Bunyavirus</i>	Tahyna	Czech Republic	Mixed	(Halouzka et al., 1991)
<i>Nairovirus</i>	Dugbe	Nigeria	Mixed	(Lee, 1979)
	Nairobi sheep disease	Kenya	<i>C. tororensis</i>	(Davies et al., 1979)
	Crimean-Congo Hemorrhagic fever [#]	Nigeria	Unidentified	(Lee, 1979)
<i>Vesiculovirus</i>	Vesicular stomatitis Indiana [#]	USA	<i>C. sonorensis</i>	(Walton et al., 1987)
<i>Flavivirus</i>	West Nile [#]	USA	<i>C. sonorensis</i>	(Naugle et al., 2004)
		USA	<i>C. arboricola</i> <i>C. biguttatus</i>	(Sabio et al., 2006)
			<i>C. stellifer</i>	

^{*} Pathogenicity uncertain in humans.

[#] World organization for animal health (OIE) listed pathogen.

loss of approximately 20% of summer working days through persistent attacks during chainsaw refueling and rest breaks in the forest districts of Kintyre, Lochaber and Wester Ross (Hendry and Godwin, 1988).

A majority of common and abundant mammalophilic *Culicoides* species in Europe have also occasionally been recorded biting humans and these studies have been significantly expanded with the recent advent of reliable polymerase chain reaction based assays for host differentiation (Garros et al., 2011; Santiago-Alarcon et al., 2012a). These species include all the primary vectors implicated in transmission of livestock arboviruses in this region: *C. obsoletus*, *C. scoticus*, *C. dewulfi*, *C. chiopterus*, *C. pulicaris* and *C. punctatus* (Dzhafarov, 1964; Overgaard Nielsen, 1964; Santiago-Alarcon et al., 2012b; Service, 1971; Szadziewski and Kubica, 1988), with the notable exception of the major Afrotropic vector *C. imicola*. Attacks on humans invariably occur only when alternative livestock or wildlife hosts are either rare or absent and the degree to which human feeding represents a sustainable source of blood-meals for *Culicoides* populations of these species has not been defined. The few available direct surveys of biting rates inflicted by livestock-associated species on human populations have indicated either low or intermittent attack rates (Dzhafarov, 1964; Overgaard Nielsen, 1964; Szadziewski and Kubica, 1988). These rates can occasionally be increased by the removal of alternative hosts or transient increases in suitable larval habitat, particularly in species that can develop in organically enriched environments e.g. *C. nubeculosus* (Szarbo, 1966). In contrast to species inflicting biting nuisance on humans, all the primary *Culicoides* vectors of livestock arboviruses worldwide are currently believed to require a blood meal before egg production (anauto-geny), including *C. brevitarsis* in Australasia (Kettle and Campbell, 1983); *C. sonorensis* in the Nearctic (Linley and Braverman, 1986) and *C. imicola* in the Afrotropic region (Braverman and Mumcuoglu, 2009).

4. What is the current potential for *Culicoides* to play a role in the transmission of arboviruses in Europe?

In 2011 a novel pathogen, provisionally named Schmallenberg virus (SBV), was discovered in Germany in adult cattle presenting clinical signs including reduced milk yield and diarrhoea (Hoffmann et al., 2012; Garigliany et al., 2012). Subsequently, SBV was demonstrated to cause congenital deformities in calves and lambs when dams were infected in the first trimester following insemination and this has since been identified as SBV's primary impact on ruminant production (Davies et al., 2012; Elbers et al., 2012). Following detection, a range of *Culicoides* species were rapidly implicated in the transmission of SBV through a series of studies in the Netherlands (Elbers et al., 2013) and Belgium (De Regge et al., 2012). Species thought to be involved included many of those previously implicated in transmission of bluetongue virus (BTV) during unprecedented incursions into both northern and southern Europe (Carpenter et al., 2009; Purse et al., 2005). Before these excursions into northern Europe, the risk of BTV infection causing clinical disease in humans was known to be negligible, and it subsequently was rapidly dismissed in discussions in the public domain. In contrast, the novel nature of SBV led to difficulties in immediately assessing the probability and consequences of human exposure (Ducomble et al., 2012).

From phylogenetic characterization, it was inferred that SBV shares a close relationship with other arboviruses that were not known to cause appreciable clinical disease in humans, including Shamonda, Aino and Akabane viruses (Doceul et al., 2013; Reusken et al., 2012). While this information was useful in informing risk assessments, it was clear that policy makers were unsure about

the degree of confidence that could be assigned to a low risk of pathogenicity inferred on this basis (Ducomble et al., 2012; Eurosurveillance Editorial, 2012; Reusken et al., 2012). Subsequently workers who were classified as being at high risk of exposure to SBV (based upon farm work and direct contact with SBV-infected animals, rather than direct knowledge of *Culicoides* biting habits) were screened using serological assays in several countries (Ducomble et al., 2012; Eurosurveillance Editorial, 2012; Reusken et al., 2012). To date, these studies have not found any evidence of human infection. It is clear, however, that the potential role of *Culicoides* in transmitting arboviruses both to and between humans in Europe has not been considered in detail from an entomological perspective (Reusken et al., 2012). In order to gain a clearer understanding of the likelihood of transmission by *Culicoides* of arboviruses both to and between humans it is therefore necessary to consider both the likelihood of future incursions and their potential for wider-scale spread in this context.

5. Routes of introduction of *Culicoides*-borne arboviruses into Europe

The routes by which *Culicoides*-borne arboviruses can be introduced into new ecosystems have been reviewed in detail, particularly with reference to the BTV-8 outbreak in northern Europe (Carpenter et al., 2009; Mintiens et al., 2008; Napp et al., 2013). Most commonly, incursions arise from the wind-assisted movement of infectious *Culicoides* midges (Burgin et al., 2013; Mellor and Wittmann, 2002; Sellers, 1992) or imported viraemic livestock (Sellers and Taylor, 1980) and hence are predictable in a wider sense where monitoring of occurrence is carried out and reported effectively in regions of transmission. The unlicensed use of partially-attenuated BTV vaccine strains is also relatively straightforward to trace using molecular phylogenetics and is known to have resulted in the transient appearance of BTV-6 (van Rijn et al., 2012) and BTV-11 (De Clercq et al., 2009) in Europe. While these routes can explain the majority of incursions of *Culicoides*-borne arboviruses into Europe, the method(s) of movement of BTV-8, BTV-25 and SBV into the region remain unknown (Carpenter et al., 2009; Maan et al., 2008).

During the initial stages of the BTV-8 outbreak, there was a general assumption that the incursion was precipitated by increases in global shipping of cargo, livestock, wildlife and humans, factors that have been invoked frequently to explain the emergence of other vector-borne diseases (Kilpatrick and Randolph, 2012). Circumstantial evidence that these routes of entry could be involved was initially provided by the identification of BTV-8 index cases in the Maastricht region of the Netherlands, an international transport hub for plants, animals and humans, although later studies appeared to suggest early occurrence of the virus in ruminants on farms close to national parks in Belgium (Saegerman et al., 2010). The epidemiological relevance of this conclusion in mode of introduction has not been investigated in detail.

Introduction of arboviruses such as BTV-8 could occur through the movement of infected *Culicoides* vectors associated with animal or human transport or through inadvertent inclusion with other cargoes, such as cut flowers. While only a few direct studies of *Culicoides* presence in ships have been carried out (and none of these were based in Europe), individual midges have been collected in 10 of 70 ships inspected offshore from Quinhuangdao port in China in crew accommodation (Nie et al., 2005). An understanding of *Culicoides* survival under the conditions imposed by transportation in standardized freight containers (Reiter, 2010) has not been quantified, nor are there any assessments of the frequency of such incursion events. Shipment of *Culicoides* eggs via the tire refurbishment trade, as has been demonstrated in mosquitoes (Eads, 1972),

appears unlikely as the eggs of all *Culicoides* species examined to date are highly susceptible to desiccation (Mellor et al., 2000).

An alternative route of arbovirus entry could involve the legal or illegal movement of viraemic exotic animals through the pet trade and zoological collections. The potential for the vast majority of arboviruses to replicate to transmissible levels in such hosts has not been investigated and accurate tracing of exotic pet trade imports is notoriously difficult even for legal shipments (Blundell and Mascia, 2005). In the case of OROV, risk of introduction associated with this route is unknown due to the current uncertainty regarding potential reservoir hosts and the current status of Brazil as a major center of wildlife collection (Magalhaes and Sao-Pedro, 2012). Globally, domestic and wild dogs have also been infected with BTV through use of live virus vaccines containing contaminated fetal calf serum (Akita et al., 1994) and also with African horse sickness virus via the ingestion of contaminated meat (Alexander et al., 1995). The potential for onwards transmission of arbovirus in these cases has not been investigated in either studies of viraemia or association with *Culicoides*, but sustained circulation by this route is thought to be unlikely (Alexander et al., 1995). The wider question of how to screen biological medicinal products used in both human and veterinary roles, together with the cell substrates used for their manufacture could become a major future consideration given increased globalization of trade (Marcus-Sekura et al., 2011; Paty, 2013).

The global movement of viraemic humans could also be envisaged as presenting a theoretical risk for introduction of OROV or novel human-to-human *Culicoides*-transmitted arboviruses. Cases of mosquito-transmitted arbovirus infection in both tourists and returning overseas workers are commonly recorded in Europe (Eisenhut et al., 1999; Harvala et al., 2009; Jelinek et al., 2002), but rarely lead to further transmission, as only restricted areas of human habitation support large vector populations. It is clear, however, that even individuals demonstrating the non-specific clinical symptoms of OROV infection would be highly unlikely to be detected during transit or at borders.

Phylogenetic studies have demonstrated that the origin of the BTV-8 strain was sub-Saharan Africa (Maan et al., 2008), although the lack of strains available for comparison made fine resolution of origin impossible (and similarly hampers geographic tracing of the origin of SBV). In the case of human-to-human *Culicoides*-transmitted arboviruses in sub-Saharan Africa, epidemics of febrile illness in humans on the scale of OROV outbreaks in Brazil would be visible even against a background of high malaria or dengue prevalence. The circulation of undetected low level endemic transmission of OROV or OROV-like arboviruses in Africa, however, cannot be easily discounted. Additionally, this region supports substantial banana and plantain production and possesses *Culicoides* species occupying a similar ecological niche to *C. paraensis* in both larval habitat and human biting habits, most obviously *C. fulvithorax* (Agbolade et al., 2006; Glick, 1990). Sub-Saharan Africa has also provided many of the isolates of zoonotic arboviruses from *Culicoides* recorded to date (Table 2) and these could be imported directly into Europe via individuals or animals with transmissible infections.

6. Establishment and spread of *Culicoides*-borne arboviruses in Europe

Following introduction, the onward transmission and establishment of a *Culicoides*-borne arbovirus in Europe would require fully infected *Culicoides* to locate and bite susceptible hosts on release from shipments, or would require viraemic hosts to come into contact with sufficiently abundant local biting populations of susceptible *Culicoides* adults to permit transmission. The survival

of imported exotic *Culicoides* and the proximity of susceptible hosts capable of developing a transmissible viraemia to points of incursion is likely to be a major determinant of the probability of successful establishment of arboviruses in a new area. Indirect evidence of indigenous *Culicoides* developing in close proximity to and feeding on exotic animals has been provided from studies of zoos, both globally and in Europe (Labuschagne et al., 2007; Nelder et al., 2010; Vilar et al., 2011). However, the vector competence of European *Culicoides* species for OROV and zoonotic arboviruses is currently unknown, however, and would significantly influence their onwards transmission potential. Assuming that the major human and livestock-associated species have at least a degree of competence for the introduced arbovirus, several aspects of the biology of these species would then be expected to influence the likelihood of onwards transmission and spread, such as habitat preference and population structure.

In terms of high abundance and rate of human contact, the most likely current candidate for sustained human-to-human transmission of arboviruses in Europe is *C. impunctatus*. This is particularly true in areas where larval habitat of *C. impunctatus* overlaps with permanent human populations in Scotland (Fig. 2). Several uncertainties underlie this assumption, however, and remain to be addressed. Firstly, the expression of autogeny in this species would require a female to survive to complete at least three reproductive cycles for transmission between hosts to occur (rather than the two required for anautogenous species). From mathematical models, vector survival and length of the period between successive blood meals are known to be major determinants of the probability of arbovirus transmission (Gubbins et al., 2008; Macdonald, 1957) and, in *Culicoides*, both parameters are impacted (in opposite directions) by temperature. A lack of reliable and straightforward age grading techniques for the *Culicoides* genus as a whole has meant that the proportion of autogenous females surviving to produce a third egg batch is not reliably known for *C. impunctatus*. Preliminary studies conducted on other autogenous *Culicoides* worldwide (Kettle, 1977; Mirzaeva, 1974), however, suggest this proportion is small (4–5% for the first anautogenous cycle) and may preclude high rates of arbovirus transmission.

A second major argument against *C. impunctatus* sustaining person-to-person transmission of arboviruses lies in uncertainty regarding the degree of ecological separation from urban or semi-urban human populations. The populations of *C. paraensis* responsible for OROV transmission appear to be unique within the genus worldwide in exploiting semi-urban habitats in close proximity to areas of high human density with few alternative feeding opportunities. Coincidence of *C. impunctatus* larval habitats and human population density in Scotland remains poorly characterized, but it appears that this species is less closely associated with these areas than *C. paraensis* in epidemic areas of Brazil, although sustained biting in garden habitats within Scotland does occur. While wide-scale surveys have been conducted for this species across Scotland (Purse et al., 2012), these were largely aimed at defining presence on farms in the role of transmitting BTV and no standardized attempt has been made to understand human contact rates in semi-urban or urban areas.

A third potentially limiting factor in epidemics driven by *C. impunctatus* is their relatively short seasonal appearance as adults in comparison to *C. paraensis*, which in Brazil can be active throughout the year (Hoch et al., 1990). Peak *C. impunctatus* activity occurs during May and June when measured by landing rates on humans (Service, 1969), by collections from black cloth hung at dusk (Hill, 1947) and from suction or light-suction trap surveys (Blackwell et al., 1992; Holmes and Boorman, 1987; Service, 1968; Takken et al., 2008). While a second peak of *C. impunctatus* activity during September has been recorded in Scotland, suggesting the production of two broods per year (Blackwell et al., 1992),

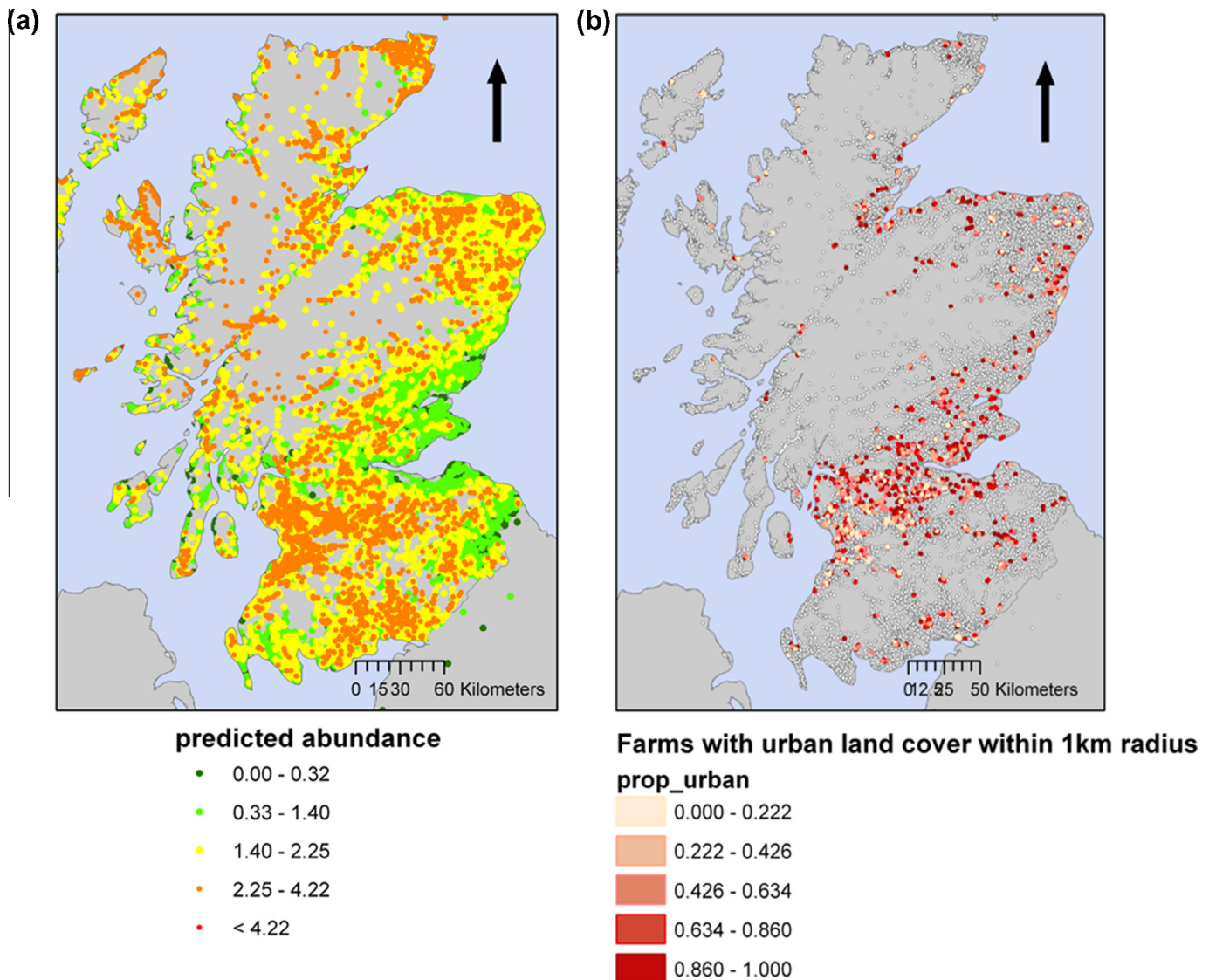


Fig. 2. Overlap of *Culicoides* with farmland and urban areas in Scotland (a) Overlap of livestock associated and human-biting *Culicoides*. Abundance of *C. impunctatus* across farms in Scotland predicted from annual mean and amplitude of temperature (daytime MODIS LST), cover of pasture and cattle densities found in a 1 km buffer zone (reproduced with permission from Purse et al. 2012) (b) Overlap of farmland and urban areas in Scotland. Distribution of all farms is shown in light grey. Around 1300 farms in the region contain urban land cover within a 1-km radius. These farms are categorized as having low to high percentage cover of urban land within the 1-km buffer zone, as indicated by the color scale from white to dark red.

there is evidence that the adult population is also curtailed earlier than that of *C. obsoletus* (Holmes and Boorman, 1987). It is additionally clear that the seasonal period during which arbovirus replication could occur in *Culicoides* will be significantly more restricted than in the Neotropics, although thermal limits to this process, driven by the extrinsic incubation period of the virus, have not been defined for OROV. Overall assessments of climate suitability for transmission of OROV in Europe have also not been carried out to date.

While major epidemics of arboviruses driven by *Culicoides*-borne transmission between humans currently appear unlikely in Europe, the potential for *Culicoides* to cause spill-over of zoonotic arboviruses from livestock and wildlife reservoirs into human populations is less straightforward to assess. In addition to the aforementioned lack of information regarding vector competence, no systematic studies of *Culicoides* biting rates on humans in proximity to farm livestock and wild ruminants have been carried out in Europe. Primary candidates for this role would include high-abundance species with generalist host preference and an association

with farm or stable holdings, most obviously *C. obsoletus*, the so-called 'garden midge' (Calvo et al., 2012; Garros et al., 2011; Lassen et al., 2012). It is also possible that the wide host preference and abundance of *C. impunctatus* may facilitate this species acting in a bridge-vector role between animal hosts and humans. Hence, areas where *C. impunctatus* larval development overlaps with farmland may also represent a higher risk of transmission of zoonotic pathogens (Fig. 1).

In addition to incursions of exotic *Culicoides*-borne arboviruses, there is also an unknown potential for emergence of currently circulating, but undetected pathogens. The drivers for this process in the case of other vector groups have recently been reviewed in detail (Kilpatrick and Randolph, 2012). From recent events it appears highly likely that apathogenic or low pathogenicity *Culicoides*-borne livestock arboviruses are currently circulating undetected in Europe. A relevant example was the discovery in Europe of Toggenburg virus (BTV-25), a strain of BTV that has low pathogenicity for livestock, which was detected in Switzerland in 2008 during routine surveillance for the highly pathogenic

BTV-8 strain in goats (Hofmann et al., 2008). In contrast to both SBV and BTV-8, where incursion timelines and spread could be at least partially traced through occurrence of clinical cases underpinned by serological surveys, both the length of time that BTV-25 has been circulating in Europe and its current distribution remain poorly explored.

From current evidence, it is highly unlikely that novel *Culicoides*-borne endemic arboviruses are circulating and causing significant levels of clinical disease in human populations in Europe. While unexplained fever and encephalitis do sporadically occur in humans in this region, localized and epidemiologically linked outbreaks of person-to-person transmission would remain visible against this limited background of cases. At present it is difficult to discount that apathogenic or very low pathogenicity strains may be transmitted between humans or from livestock to humans by *Culicoides*. An argument against their occurrence, however, is that during vector competence studies of *Culicoides* in Cyprus (Mellor and Pitzolis, 1979) and Italy (Caracappa et al., 2003; Savini et al., 2005) only BTV strains already in circulation were detected, despite using diagnostic techniques and assay systems with a broad range of detection (blind passage through chick embryos and vertebrate cell lines). Similarly, more general surveys of arboviruses in wildlife and a wide variety of sentinel hosts have failed to uncover evidence of widespread circulation of unknown arboviruses (Gratz, 2006; Hubalek and Halouzka, 1996; Lundstrom, 1999).

7. Veterinary and public health responses to *Culicoides*-borne arbovirus incursions

Following identification, control strategies used to reduce further spread of *Culicoides*-borne arboviruses of livestock could include euthanizing index cases; imposing trade movement restrictions; using a variety of techniques to reduce *Culicoides*-host contact and compulsory or voluntary vaccination of livestock hosts to either eradicate the pathogen or reduce clinical disease. At present the degree to which systematic eradication plans are considered is dependent upon the probable economic impact of arbovirus outbreaks, the potential for an arbovirus to persist in particular regions of Europe (as this region includes areas where *Culicoides* adults are absent for significant periods over winter) as well as the technical and financial challenges posed in production of a suitable vaccine. In the case of BTV-8, despite early evidence of high pathogenicity (Darpel et al., 2007), an apparent means of overwintering (Darpel et al., 2009; De Clercq et al., 2008; Wilson et al., 2008) and the availability of technology to produce a highly effective vaccine (Parker et al., 1975), it still required between eighteen months and 2 years to deploy systematic vaccination campaigns, partly due to the need to identify a large enough market before production could commence. In the case of SBV clinical impact is currently thought to be relatively limited and there is a potential that the virus may eradicate itself from large areas due to rapid and efficient transmission resulting in antibody protection in a high proportion of hosts. In addition, SBV originates from a virus group which is not usually considered sufficiently economically important to warrant systematic vaccination (though changes in host management to prevent exposure of pregnant females to infected vectors during critical periods of foetal development may be cost-effective).

Following detection of a human-to-human *Culicoides*-borne arbovirus in Europe, the public health response would be determined by similar drivers to livestock pathogens but with a greater emphasis on clinical impact. A key consideration in southern Europe would be the potential of established major nuisance biting species of mosquito to act as agents of transmission, because *Culicoides*

populations in this region would be highly unlikely to sustain even small-scale outbreaks as the fauna in this region is dominated by *C. imicola*, which is allegedly highly zoophilic (Calvete et al., 2008; Conte et al., 2009). The contribution of the invasive *Stegomyia albopicta* in particular is likely to be important given its demonstrable ability to sustain outbreaks of chikungunya virus between humans at least transiently in Italy (Talbalaghi et al., 2010). Testing of a Brazilian population of this species with OROV, however, led only to very low rates of infection and limited dissemination (Smith and Francy, 1991).

The control of *Culicoides* has previously been reviewed in detail for Europe (Carpenter et al., 2008) and there are additional highly informative historical reviews of attempts to control biting nuisance from *C. impunctatus* in the Scottish highlands using insecticidal application (Blackwell, 2001; Kettle, 1996; Stuart et al., 1996). In both livestock and human-associated species, wide-scale control of larvae or adults through treatment, removal or covering of development or resting sites is considered unfeasible due to the broad range and abundance of habitats utilized (Carpenter et al., 2008). Research for preventing biting of *C. impunctatus* on human hosts has therefore largely centered upon the use of repellents, of which the current gold standard is *N,N*-Diethyl-meta-toluamide (DEET) (Carpenter et al., 2008; Corbel et al., 2009). Additional alternative active ingredients have also been investigated including eucalyptus (Trigg, 1996); Icaridin (Carpenter et al., 2005); salicylic acid (Stuart et al., 2000) and azadirachtin (Blackwell et al., 2004). All of these repellents have been shown to provide at least some degree of protection during transient attacks (e.g. during tourist activities). These studies of existing repellents have also been complemented by the identification of novel volatile chemicals from humans that interrupt host-location by *C. impunctatus* and may be useful in the future design of dedicated repellents for this species (Logan et al., 2009).

For individuals exposed to persistently high biting rates repeated application of repellents becomes unfeasible due to dermatological reactions, and treated clothing and mechanical barriers such as netted hoods may provide more convenient protection (Dever et al., 2011; Harlan et al., 1983; Hendry, 2011). In the case of forestry workers, this approach has been tried successfully in several areas of Scotland (Hendry and Godwin, 1988), although the rate of use is dependent on a variety of factors, not least the tolerance towards biting of the individual concerned. Following incursion of an arbovirus and associated education, this rate would be likely to increase both in forest workers and other human populations exposed to *Culicoides* biting attacks.

In addition to repellents, traps baited with natural repellents (semiochemicals) also demonstrate some promise in reducing incidence of adult host-seeking *C. impunctatus*, particularly where the nuisance population is of limited abundance. Dedicated lures for this purpose have been developed (Mands et al., 2004) and commercial traps have been produced, although the efficacy of these in reducing overall *Culicoides* biting rates (and hence arbovirus transmission risk) has not been quantified in Europe. To date, lures have not been developed for livestock-associated *Culicoides* species, although preliminary studies have been conducted with generic attractant compounds that show promise (Harrup et al., 2012). In the case of *C. impunctatus*, these techniques are unlikely to lead to permanent reductions in population abundance due to autogeny and huge source populations, but they may impact on the major species associated with livestock, which are largely anautogenous.

In the event of an incursion of an arbovirus into Europe that is capable of person-to-person spread by *Culicoides* midges, education is likely to play a key role in promoting avoidance of areas supporting substantial populations of vectors. The substantial nuisance already inflicted by *C. impunctatus* has led to the development of a 'midge forecast' for tourists and local inhabitants in

Scotland which is disseminated via newspapers, a website (<http://www.midgeforecast.co.uk/>) and most recently a mobile phone application. Combined with data concerning *C. impunctatus* distribution and fine-scale habitat use, the midge forecast could be usefully employed to warn of geographical areas and habitats of high exposure risk. A clearer understanding of which recreational activities and jobs require prolonged exposure to *Culicoides* would be extremely useful in qualitatively assessing risk of exposure. Overlap on farms between *Culicoides* populations and human workers is more difficult to assess, however, and may be significantly influenced by husbandry practices. For example, it is quite possible that those involved in forestry or game-related activities in proximity to farms may suffer greater exposure than the farmers themselves.

8. Conclusions and key knowledge gaps

Culicoides are among the most abundant vectors of arboviruses found in Europe, but current evidence demonstrates that their impact on human health in this region is currently limited to biting nuisance. However, the existence of one or more proven, but as yet undescribed, route of entry for *Culicoides*-borne arboviruses into Europe raises the potential of future impacts on human health. From reviewing current knowledge of *Culicoides* populations both in Europe and in areas of arbovirus transmission worldwide we reach the following conclusions:

1. Future introduction of known or unknown arboviruses that are transmitted in epidemics between humans by *Culicoides* (OROV) are unlikely to lead to sustained outbreaks of disease in Europe without the involvement of additional vector groups and/or as yet unknown reservoir hosts.
2. The role of *Culicoides* in the transmission of known and unknown zoonotic arboviruses worldwide is currently unclear, but thought to be limited. Nevertheless, following an incursion, spillover transmission from livestock populations into humans driven in Europe by *C. obsoletus* and/or *C. impunctatus* currently cannot be discounted and should be included for consideration in risk assessments.
3. The likelihood that changes in the pathogenicity or prevalence of endemically circulating *Culicoides*-borne livestock arboviruses in Europe could change such that they affect human beings is considered to be of low probability. The likelihood that unidentified zoonotic or human-to-human transmitted arboviruses are currently circulating unnoticed in Europe is similarly low.

The outbreak of SBV has highlighted uncertainty surrounding *Culicoides* vector roles, but has also demonstrated some of the advances in technology likely to transform future surveillance of zoonotic arboviruses in Europe. The increasing use of next-generation sequencing in a screening role across Europe will revolutionize understanding of both endemic and exotic circulation of low-pathogenicity arboviruses, driven initially by investigations of unexplained clinical cases in affected hosts (Delwart, 2007; Radford et al., 2012). The broad-scale sensitivity of next generation sequencing techniques will allow increasing use of sentinel surveillance worldwide by reducing cost/benefit ratios that currently make such schemes unworkable. These data are likely to be complemented in due course by analyses of the entire viral populations of *Culicoides* themselves, an area that has yet to be explored with next-generation sequencing methodologies, but which has already shown promise in identifying novel virus species and strains in mosquitoes (Bishop-Lilly et al., 2010).

In addition, detailed serological surveys of arboviruses currently being conducted for SBV on humans in Europe may prove useful in predicting points of contact between vectors and hosts if a

zoonotic *Culicoides*-borne arbovirus emerges in Europe. Screening for potential clinical disease or seroconversion in human populations should be targeted towards geographic areas that include overlap between *C. impunctatus* and *C. obsoletus* populations, as preliminarily characterized for Scotland (Purse et al., 2012). A greater understanding of the degree of exposure of humans to *Culicoides* biting in Europe during both work and recreation would complement these studies. There is also an additional requirement to understand how the host preferences and abundance of livestock- and human-biting *Culicoides* species vary seasonally across heterogeneous suburban and recreational landscapes.

Direct examination of potential introduction routes of *Culicoides*-borne arboviruses into northern Europe would be helpful in providing a framework for risk assessment (Napp et al., 2013). If specific cargoes could be defined as presenting a particular risk of containing *Culicoides*, this would allow analysis of import patterns and habitat of origin and destination ports facilitating inference regarding species of arbovirus that could potentially be introduced (Tatem and Hay, 2007; Tatem et al., 2006). Similarly, tracing of human movements into Europe from areas of endemicity have already demonstrated utility in mosquito-borne arbovirus research and could be usefully extended to monitor the risk of emerging *Culicoides*-borne infections (Tatem and Hay, 2007).

Two major areas of uncertainty exist in the degree of vector competence of *Culicoides* species present in Europe for human-pathogenic arboviruses and the ability of available livestock and wildlife hosts to replicate these arboviruses to transmissible levels. To date, no studies of infection of abundant human and livestock biting species of *Culicoides* in Europe have been carried out with arboviruses transmitted from animals to humans or among humans. This is in part due to the challenging technical difficulties of feeding these species, although techniques to allow screening of populations have been developed for both *C. obsoletus* (Carpenter et al., 2006; Venter et al., 2005) and *C. impunctatus* (Blackwell et al., 1994b). While these studies are unlikely to be prioritized above more obvious mosquito vectors in the case of known zoonotic arboviruses, they may assist in more detailed assessments of the probability of disease establishment. Assessing the potential for development of transmissible viraemia in livestock or wildlife, however, is far more straightforward to assess in areas of endemic circulation as part of detailed and prioritized epidemiological investigations. These studies are vital in promoting a worldwide understanding of patterns of virus transmission and their neglect, particularly in resource-poor areas where other aspects of public health may be prioritized, has obvious implications in an unprecedented era of globalization.

An additional question that has also not been satisfactorily addressed in Europe as a whole is a broader understanding of how the diverse range of potential vector groups that exist in this region may interact in transmission roles. This is particularly evident in the case of *Culicoides*, which are considered by a large proportion of the entomological community to be only of relevance to livestock arbovirus transmission. In light of repeated calls for a “One Health” approach uniting veterinary and medical expertise, coherent ecologically-based surveillance taking into account those multiple vector groups and wild hosts present at locations across Europe, appears to be a desirable goal. While this will require an array of expertise and sharing of datasets, it is likely to greatly improve understanding of transmission of arboviruses and lead to a clearer understanding of the risk of emergence and sustained circulation of arboviruses in Europe.

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